

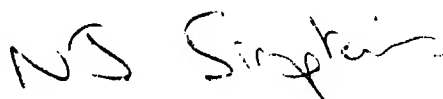
## **DECLARATION**

RWS Group Ltd, of Europa House, Marsham Way, Gerrards Cross,  
Buckinghamshire, England,

hereby declares that one of its translators is conversant with the English and French  
languages and is a competent translator thereof.

RWS Group Ltd further declares that the following is a true and accurate translation  
into English of the French Patent Application N° 02/04,926 filed on April 19, 2002.

Signed this 4th day of July 2008

A handwritten signature in black ink, appearing to read 'NT Simpkin', written in a cursive style.

N. T. SIMPKIN  
Deputy Managing Director - UK Translation Division  
For and on behalf of RWS Group Ltd

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Head of Patent Division

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**APPLICATION FOR ISSUE OF CERTIFICATE** 1/2  
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<b>3 TITLE OF INVENTION (no more than 200 characters or spaces)</b> <b>ANTI-HIV COMPOSITION, METHOD OF PRODUCTION AND MEDICINAL PRODUCT.</b>			
<b>4 STATEMENT OF PRIORITY OR REQUEST TO BENEFIT FROM FILING DATE OF A PRIOR FRENCH APPLICATION</b>		Country or organization Date [ .... / .... / ..... ] N°  Country or organization Date [ .... / .... / ..... ] N°  Country or organization Date [ .... / .... / ..... ] N°  <input type="checkbox"/> In the event of other priorities, tick the box and use "Continuation" sheet	
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The inventors are the applicants		<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No In this case provide a separate inventor designation	
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**DESIGNATION OF INVENTOR(S) Page n° 1... / 2...**  
 (If applicant is not the inventor or the sole inventor)

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<b>Your references for this file</b> (optional)		B13987.3 EE BD1388/CNRS	
<b>NATIONAL REGISTRATION N°</b>		02.04926 of 19.04.2002	
<b>TITLE OF THE INVENTION (no more than 200 characters or spaces)</b>  ANTI-HIV COMPOSITION, METHOD OF PRODUCTION AND MEDICINAL PRODUCT.			
<b>THE APPLICANT(S):</b> COMMISSARIAT A L'ENERGIE ATOMIQUE 31/33 rue de la Fédération 75752 PARIS 15 CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE 3 rue Michel Ange 75794 PARIS CEDEX 16			
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surface. This second interaction, gp120/CCR5 or  
gp120/CXCR4, then results in a reorganization of the  
gp120/gp41 protein complex. This reorganisation exposes  
gp41, which then allows initiation of the fusion of the  
5 cell and viral membranes, and entry of the viral  
genetic material into the cell.

These studies make it possible to define two novel  
therapeutic targets: inhibition of the interaction of  
10 gp120 with CD4 and CCR5 or CXCR4, and inhibition of the  
fusion [3].

#### **Prior art**

15

The references between [] refer to the reference list  
attached in the appendix.

In the field of human immunodeficiency virus (HIV)  
20 infection, tritherapies associating nucleoside  
inhibitors, non-nucleoside inhibitors and/or  
antiproteases ("HAART" for "Highly Active  
Antiretroviral Treatment") target the replication and  
maturation of the virus.

25

These treatments make it possible to substantially  
reduce the viral load, but they do not make it possible  
to totally eradicate the virus from the body. In fact,  
if the taking of medicinal products is stopped, even  
30 after several years of treatment, this invariably  
results in a rapid increase again in the viral load in  
the plasma. Besides this disadvantage, these treatments  
are considerably toxic and have many side effects.

In the context of the search for new treatments against AIDS, the processes of adsorption of the virus onto the host cell constitute a particularly attractive therapeutic target due in particular to the fact that  
5 this step takes place outside the cell.

Peptides which bind to gp41 and which inhibit its fusion activity have been developed [4, 5]. The clinical studies currently in progress give positive  
10 results, indicating that inhibition of the fusion, and therefore of the entry of the virus, effectively corresponds to an advantageous therapeutic target.

As regards the attachment of the virus, various studies  
15 have explored the use of soluble CD4 for inhibiting the interaction of the virus with the CD4 expressed at the surface of cells that are targets for HIV. This solution has proved to be ineffective, because, in binding to the virus, the soluble CD4 exposes the CD4i  
20 epitope and in fact promotes the interaction of the virus with the CCR5 or CXCR4 coreceptor, which, in certain cases, increases infection [6].

In addition to CD4, the coreceptors are also sites of  
25 attachment of the virus to the cells. The natural ligands for these coreceptors are chemokines, in particular RANTES and MIP for CCR5, and SDF for CXCR4. In vitro or on cells in culture, these chemokines inhibit the interaction of the virus with the cells [7,  
30 8], but also induce a certain number of cell responses making them difficult to use from a therapeutic point of view. A certain number of compounds such as AMD301 or peptides which bind to the coreceptors also have antiviral effects [9, 10]. However, in targeting the  
35 HIV coreceptors, these various molecules also block the intrinsic functions of the cell linked to the use of these coreceptors.



Besides these cell receptors, HIV is capable of binding to other molecules present on the cells that it infects, such as DC-SIGN, sphingolipids or heparan sulphates [11].

Heparan sulphates are complex polysaccharides belonging to the glycosaminoglycan (GAG) family. They are abundantly present at the cell surface and in interstitial matrices, where they are found anchored to the extracellular domain of specific glycoproteins, heparan sulphate proteoglycans (HSPGs). Heparan sulphates (HSs), which were discovered half a century ago from preparations of heparin (another type of GAG having very similar properties), differ from any other biological macromolecule by virtue of the diversity of their structure and of the functions that they exercise. They are capable in particular of binding HIV gp120, and the virus uses this property in order to adsorb to the surface of target cells. The site of interaction of heparan sulphates on gp120 is located on a variable structure, called V3 loop [12]. However, the exact role of these polysaccharides during infection with HIV remains relatively unclear. Studies have shown that elimination of the heparan sulphates expressed at the surface of cells contributes to making them less permissive to infection with the virus [11], demonstrating the importance of this molecule for the attachment and the entry of the virus.

On the basis of these observations, various polyanionic molecules of the heparin type have been developed in order to inhibit the interaction of the virus with the cells. However, the first clinical trials have shown only little or no activity of these molecules, and it has been possible to observe toxic effects in certain cases [13, 14].

It therefore appears to be necessary to develop new treatments against AIDS that are less restricting, result in fewer side effects and make it possible to  
5 avoid evasion of the treatment, i.e. the appearance of resistant viruses which no longer respond to the treatments. It is also necessary to find other anti-HIV therapies that are directed against new targets.

10 It is in this context that the inventors have produced the present invention.

#### **Disclosure of the invention**

15

The aim of the present invention is precisely to overcome the abovementioned disadvantages by providing a novel composition which can be used as an anti-HIV agent. This composition is capable of blocking the  
20 entry of the AIDS virus into its host cells. In this respect, it can be used for preparing a medicinal product, in particular a medicinal product intended for the treatment of AIDS.

25 The composition of the present invention is characterized in that it comprises a polyanion and a molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein.

30 Thus, in accordance with the present invention, the inventors have combined, within a single composition, firstly, a polyanion, for example of the heparin or heparan sulphate type and, secondly, a molecule capable of inducing the exposure of the CD4i epitope of the  
35 gp120 viral protein, for example of a soluble CD4 peptide. They have shown that this composition makes it possible to inhibit, unexpectedly, both the virus-cell

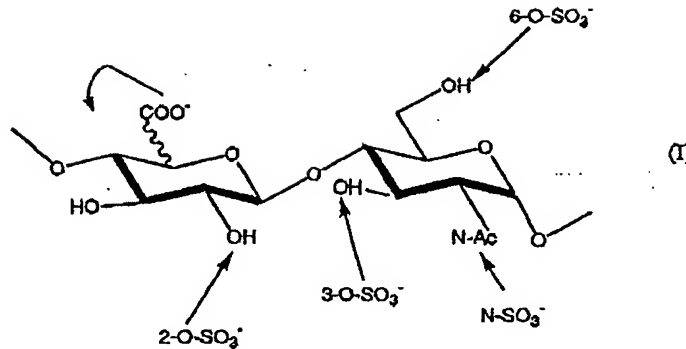
membrane heparan sulphate interaction, by blocking the V3 loop, and the virus-coreceptor interaction, by blocking the CD4i site. In fact, the inventors have shown that there are actually two domains or sites of interaction of heparin- or heparan sulphate-polyanions on gp120. The first is the V3 loop, the second is the CD4i domain. They have shown (see examples below) that heparin, or heparin fragments of sufficient size, in the presence of a CD4 peptide, interacts with the CD4i domain of the gp120 viral protein and that this combination greatly inhibits the gp120/48d or 17b antibody interaction. 48d or 17b are used as mimics of coreceptors.

This blocking of HIV with the composition of the present invention is all the more unexpected since those skilled in the art are aware that the CD4 molecule used alone can have the reverse effect of that desired, since it exposes the domains for interaction with the coreceptors, and can therefore increase the virus infectivity.

The composition of the present invention is therefore directed towards a novel therapeutic target by means of heparin or other polyanions in the presence of the CD4 peptide, namely the blocking of the interaction of HIV with its coreceptors. This solution is very advantageous, from the therapeutic point of view, for inhibiting the attachment of the virus to the cells, since it targets the virus itself and not the cells. It is therefore, firstly, free of the cellular effects which are observed with the products of the prior art when it is the coreceptors that are targeted. In addition, the toxicity of the composition of the present invention for an organism is less than most of the chemical compounds of the prior art due to the nature of its constituents.

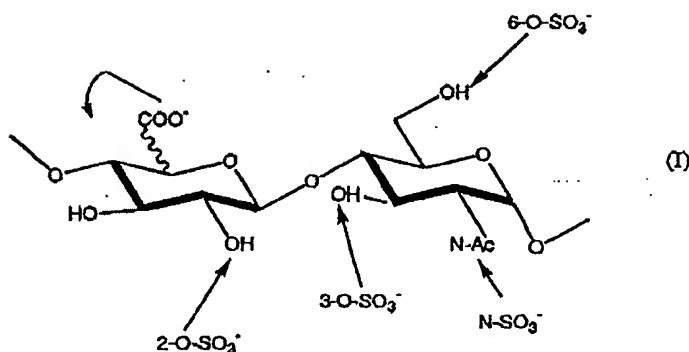
According to the invention, the polyanion may advantageously be chosen from the group consisting of heparin, heparan sulphate, and a polyanion equivalent  
5 to heparin or to heparan sulphate. It is, for example, Dextran sulphate (commercial name, Ueno fine chem), Curdlan sulphate (commercial name, Ajinomoto), 2-Naphthalenesulphonate polymer (commercial name, Procept), Pentosan polysulphate (commercial name, Baker  
10 norton pharm; Hoechst), or Resobene (commercial name).

The structure of the constituent disaccharide (basic element) of the heparin and of the heparan sulphate according to the present invention is of formula (I)  
15 below:



5 It is preferable for the polyanion not to be too long, since it would have an anticoagulant activity, which is not desired in the present invention, and would form aspecific bonds with various proteins, in particular thrombin or antithrombin III. Its length will  
10 preferably be similar to a heparin chain having a degree of polymerization as defined below. The polyanion preferably has at least two anionic groups per disaccharide. According to the present invention, when the polyanion is heparin or heparan sulphate, it  
15 will preferably have a degree of polymerization dp of 10 to 24, advantageously of 12 to 24, preferably of 16 to 22.

According to the invention, the polyanion may be  
20 prepared by partial depolymerization of heparin or of heparan sulphate by means of an enzymatic method, for example by means of heparinase, or a chemical method, for example by means of nitrous acid. When they are obtained chemically, the heparans may be defined by the  
25 presence of N-sulphated or N-acetylated glucosamine, or glucosamine not substituted in the N-position, linked to a uronic acid (glucuronic acid or iduronic acid) with a variable proportion of sulphate group. Structural mimics of



It is preferable for the polyanion not to be too long, since it would have an anticoagulant activity, which is not desired in the present invention, and would form aspecific bonds with various proteins, in particular thrombin or antithrombin III. Its length will preferably be similar to a heparin chain having a degree of polymerization as defined below. The polyanion preferably has at least two anionic groups per disaccharide. According to the present invention, when the polyanion is heparin or heparan sulphate, it will preferably have a degree of polymerization dp of 10 to 24, advantageously of 12 to 24, preferably of 16 to 22. According to the invention, the heparin, the heparan sulphate or the polyanion equivalent to heparin or heparan sulphate may have a degree of polymerization dp of 12 to 20, for example of 15 to 17.

According to the invention, the polyanion may be prepared by partial depolymerization of heparin or of heparan sulphate by means of an enzymatic method, for example by means of heparinase, or a chemical method, for example by means of nitrous acid. When they are obtained chemically, the heparans may be defined by the presence of N-sulphated or N-acetylated glucosamine, or glucosamine not substituted in the N-position, linked to a uronic acid (glucuronic acid or iduronic acid) with a variable proportion of sulphate group. Structural mimics of

these oligosaccharides may be obtained by chemical synthesis.

According to the invention, the molecule capable of  
5 inducing the exposure of the CD4i epitope of the gp120  
viral protein can be chosen from a CD4 peptide or a  
derivative of this peptide, or else a monoclonal  
antibody which binds to the gp120 viral protein and  
which is capable of activating said gp120 protein in a  
10 manner equivalent to the CD4 peptide.

When it is a CD4 peptide, it is preferably soluble for  
the obvious reasons of facilitation of its interaction  
with the gp120 viral protein in liquid medium, and of  
15 facilitation of its access to its target.

According to the invention, the CD4 peptide  
advantageously has the sequence (I) below:

20 Cys or TPA - P<sup>1</sup> - Cys - P<sup>2</sup> - Cys - P<sup>3</sup> - Cys - Ala or Gln  
- Gly or (D)Asp or Ser - Ser or His or Asn - Xaa<sup>J</sup> - Cys  
- Thr or Ala - Cys - Xaa<sup>K</sup> - NH<sub>2</sub>

in which TPA represents thiopropionic acid, Xaa<sup>J</sup>  
25 represents  $\beta$ -naphthylalanine, phenylalanine or  
biphenylalanine, Xaa<sup>K</sup> represents Gly, Val or Ileu, P<sup>1</sup>  
represents 3 to 6 amino acids, P<sup>2</sup> represents 2 to 4  
amino acids and P<sup>3</sup> represents 6 to 10 amino acids, the  
amino acids in P<sup>1</sup>, P<sup>2</sup> and P<sup>3</sup> being natural or unnatural,  
30 identical or different, and P<sup>1</sup>, P<sup>2</sup> and P<sup>3</sup> possibly  
having a common sequence, said peptide having a  
 $\beta$ -hairpin conformation in which the  $\beta$ -turn is made up  
of the amino acid residues Ala or Gln - Gly or DAsp or  
Ser-Ser or His or Asn- Xaa<sup>J</sup> of its sequence (A). In  
35 fact, these peptides show a very great affinity for the  
gp120 viral protein.

Examples of such peptides which can be used in accordance with the present invention are the peptides of sequences ID No. 1 to ID No. 18 of the sequence listing attached in the appendix, or equivalent  
5 peptides.

These peptides can be prepared by conventional techniques of solid-phase chemical synthesis or of genetic recombination.

10

When the molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein is an antibody, it can be chosen, for example, from those described in the document Sullivan N, Sun Y, Binley J, Lee J, Barbas CF 3rd, Parren PW, Burton DR, Sodroski J, Determinants of human immunodeficiency virus type 1  
15 envelope glycoprotein activation by soluble CD4 and monoclonal antibodies. *J Virol* 1998; 72(8): pp.6332-6338.

20

According to a first embodiment of the present invention, the polyanion and the molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein are mixed in said composition. This  
25 composition in accordance with the present invention makes it possible to expose the site of interaction with the coreceptors (CD4i site) and, concomitantly, to block this site by means of the oligosaccharide part consisting of the polyanion.

30

According to this first embodiment, the polyanion and the molecule capable of inducing the exposure of this CD4i epitope of the gp120 viral protein are advantageously mixed in said composition in proportions  
35 of 1 to 10 mol of polyanion per 0.5 to 1.5 mol of molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein, preferably of 5 mol



of polyanion per mole of molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein.

5 The present invention also relates to a method for producing the composition according to this first embodiment of the invention, comprising the following steps:

- preparing the polyanion,
- 10 - preparing the molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein,
- mixing the polyanion and the molecule capable of inducing the exposure of the CD4i epitope of the gp120
- 15 viral protein prepared so as to obtain said composition.

The mixture will preferably be prepared in a biological buffer so that it can be used to produce an  
20 administrable medicinal product. The pH is preferably approximately 7, and the solution contains, for example, 15 g/l of NaCl.

According to a second embodiment of the present  
25 invention, the polyanion and the molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein are linked to one another in said composition. They form a hybrid of polyanion/molecule capable of inducing the exposure of the CD4i epitope of  
30 the gp120 viral protein hybrid.

For example, according to the invention, the polyanion and the molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein are linked  
35 to one another at one of the ends of the polyanion.

When the polyanion used is short, for example with a

degree of polymerization dp of 10 to 12, it may be necessary to link the polyanion and the molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein by means of a spacer arm, in order to allow the hybrid formed to bind to all its targets on the gp120 viral protein. This may also be the case when the molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein is too short. The spacer arm may be any polymer, preferably soluble in aqueous buffers, of appropriate length. Mention may be made, for example, of polyosides or polyglycols. It may be, for example, polyethylene glycol:  $(CH_2CH_2O)_n$ . Preparations of spacer arms of this type which can be used in the present invention have been widely described in the prior art, for example in documents [18] and [19] (see attached reference list).

The present invention also relates to a method for producing the composition according to the second embodiment of the invention, comprising the following steps:

- preparing the polyanion,
- preparing the molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein,
- linking the polyanion and the molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein prepared so as to obtain said composition.

The linking of the polyanion with the molecule capable of inducing the exposure of the CD4i epitope can be formed by any techniques known to those skilled in the art, for example for linking a polyanion and a peptide. For example, the various methods described in documents [15], [16] and [17] (see attached reference list) for coupling an oligosaccharide to a polypeptide can be

used in the present invention.

According to the present invention, for the reasons mentioned above, it is also possible to use any type of  
5 bridging agent, or spacer arm, which binds, firstly, to one end of the oligosaccharide and, secondly, to a part of CD4 that is not essential to its function. The spacer arm may be one of those mentioned above. It can be prepared in the manner described in documents [18]  
10 and [19].

The hybrid molecule of the present invention has three advantages: it binds to the gp120 viral protein on the CD4 interaction site, on the V3 loop, when these gp120  
15 molecules are derived from viruses using CXCR4 as coreceptor, and on the domain of interaction with the coreceptors (CD4i domain), as shown diagrammatically in Figure 7 attached in the appendix. It therefore makes it possible to simultaneously block all the domains  
20 that gp120 uses to interact with its cell receptors and coreceptors.

Other characteristics and advantages will become apparent to those skilled in the art in the light of  
25 the examples below, given by way of non-limiting illustration, with references to the figures and sequences attached in the appendix.

#### **Brief description of the sequence listing**

30

The sequences ID No. 1 to ID No. 18 of the sequence listing attached in the appendix are non-limiting examples of molecules capable of inducing the exposure of the CD4i epitope of the gp120 viral protein for the  
35 purpose of the present invention. These molecules are peptides originating from human CD4 (Seq ID No. 1), or artificial peptides originating or derived from

scorpion venom peptides (Seq ID Nos. 2 to 18).

**Brief description of the figures**

- 5     -     Figure 1 is a graph representing the amount of  
gp120/CD4, in resonance units (RU), bound to heparin as  
a function of time t (in seconds) for various  
concentrations of CD4 (in nM): curves from bottom to  
top: 0; 50; 100; 250; 500 nM.
- 10     -     Figure 2 is a graph representing the amount of  
gp120/CD4, in resonance units (RU), bound to heparin as  
a function of the concentration of CD4 (in nM).
- 15     -     Figure 3 is a graph representing the evolution of  
the gp120 viral protein/antibody 48d interaction  
(response RU) as a function of time (in seconds) for  
various concentrations of CD4 peptide (in nM): curves  
from bottom to top: 0; 50; 100; 250; 500 nM.
- 20     -     Figure 4 is a graph representing the inhibition of  
the interaction of the gp120/CD4 complex with the  
antibody 48d (response RU) as a function of time (in  
seconds) by various concentrations of heparin H (in  
25     nM): curves from top to bottom: 0; 3; 6; 12; 30 µg/ml.
- Figure 5 is a graph representing the inhibition of  
the amount of gp120/CD4 complexes bound to 48d as a  
function of the size of the heparin fragment in degree  
30     of polymerization, from dp 0 to dp 18 (curves from top  
to bottom: dp 0; dp 2; dp 4; dp 6; dp 8; dp 10; dp 12;  
dp 14; dp 16; dp 18).
- Figure 6 is a graph representing the amount of  
35     gp120/CD4 complexes bound to 48d as a function of the  
size of the heparin fragment based on the data  
represented in Figure 5.

Figure 7 is a drawing showing diagrammatically the gp120 viral protein, and the interaction of a composition in accordance with the second embodiment of the present invention with the gp120 viral protein. In this figure, sCD4bs ("s" for soluble, "bs" for binding site) = CD4-binding site, V3 = V3 loop, CD4ni = non-induced ("ni"), (non-accessible) coreceptor-binding site, CD4i = coreceptor-binding site induced by the binding of CD4 to gp120 ("i" for induced), CD4/H = hybrid molecule of CD4 peptide/heparin or heparan sulphate according to the second embodiment of the present invention, and H = heparin or heparan sulphate.

Figures 8 a) to h) are representations in the form of graphs of the interaction of complexes of gp120 viral protein and gp120/CD4 on an activated sensorchip with heparin, at various concentrations of gp120 viral protein: 0 nM (a); 0.62 nM (b); 1.25 nM (c); 2.5 nM (d); 5 nM (e); 10 nM (f), 20 nM (g) and 40 nM (h), with preincubation (continuous lines) or without preincubation (discontinuous lines) with 80 nM of soluble CD4.

Figure 9 is a representation in the form of a graph of the inhibition of the gp120/CD4 interaction by heparin and oligosaccharides of heparin (H) on a sensorchip activated with mAb17b. The gp120 viral protein (5 nM) was preincubated successively with a CD4 peptide (10 nM) and with concentrations of heparin ([H]) at 0 nM (curve a); 2.1 nM (b); 4.2 nM (c); 8.3 nM (d); and 16.7 nM (e), before being injected onto the mAb17b surface.

Figure 10 is a representation in the form of a graph of the inhibition of the gp120/CD4 interaction by heparin and oligosaccharides of heparin (H) on the

sensorchip activated with mAb17b. The gp120 viral protein (5 nM) was preincubated successively with a CD4 peptide (10 nM) and with a concentration of heparin of 40 nM, with various degrees of polymerization: dp 0 (curve 1); dp 2 (curve 2); dp 4 (curve 3); dp 6 (curve 4); dp 8 (curve 5); dp 10 (curve 6); dp 12 (curve 7), dp 14 (curve 8); dp 16 (curve 9); dp 18 (curve 10), before being injected onto the mAb17b surface.

Figure 11 represents the absorbance at 230 nm of various fractions (F) of 15 ml obtained during an enzymatic synthesis of heparin at degrees of polymerization dp ranging from 2 to 10 (the dp corresponds to the figures indicated on the curve).

#### EXAMPLES

In the following examples, the analyses of gp120-heparin interaction were carried out by surface plasmon resonance (BIAcore system (trademark)). This technique, which makes it possible to perform real-time interaction measurements, also has the advantage of providing a model similar to the physiological reality, where the heparin immobilized on the sensorchip constitutes a two-dimensional interface like the cell surface.

#### EXAMPLE 1: SYNTHESIS OF A CD4 PEPTIDE WHICH CAN BE USED FOR PRODUCING THE COMPOSITION OF THE PRESENT INVENTION

A peptide from a sequence listing attached in the appendix is synthesized by solid-phase chemical synthesis with an Applied Biosystems automatic peptide synthesizer, mod. 433A, and by Fmoc chemistry, which uses the fluorenylmethyloxycarbonyl (Fmoc) group for temporary protection of the  $\alpha$ -amino function of the amino acids. The protective groups used to prevent the

side reactions of the amino acid side chains, in this Fmoc strategy, were tert-butyl ether (tBu) for the Ser, Thr and Tyr residues; tert-butyl ester (OtBu) for Asp, Glu; trityl (Trt) for Gln, Asn, Cys, His; tert-butylloxycarbonyl (Boc) for Lys and 2,2,5,7,8-pentamethylchroman-6-sulfonyl (Pmc) for Arg.

The coupling reaction takes place with an excess of 10 equivalents of amino acids (1 mmol) relative to the resin (0.1 mmol). The protected amino acid is dissolved in 1 ml of N-methylpyrrolidone (NMP) and 1 ml of a 1 M solution of 1-N-hydroxy-7-azabenzotriazole (HOAt) in the NMP solvent. 1 ml of a 1 M solution of N,N'-dicyclohexylcarbodiimide (DCC) is then added. After activation for 40 to 50 minutes, the active ester formed is transferred into the reactor which contains the resin. Before this transfer then coupling step, the resin is deprotected by removal of its Fmoc group with a 20% solution of piperidine in NMP. The excess piperidine is removed by washing with NMP after approximately 5 to 10 minutes.

After synthesis of the peptide, the peptide-resin is treated five times with a 2% solution of hydrazine in DMF. The coupling of a linker arm is carried out for one hour at ambient temperature in DMF with 10 equivalents of Fmoc-8-amino-3,6-dioxaoctanoic acid using the HBTU reagent in the presence of diisopropylethylamine. The Fmoc group is then deprotected with 20% of piperidine in DMF.

The peptide-resin is immediately treated with 10 equivalents of Traut reagent (2-iminothiolane hydrochloride (Sigma)) in the presence of DIEA. The peptide is finally released and deprotected as described below.

The cleavage of the resin and the cleavage of the protective groups present on the side chains were carried out simultaneously by treating the peptide linked to the resin with trifluoroacetic acid (TFA).  
5 Before performing the cleavage, the resin was washed several times with dichloromethane (DCM) and finally dried. The reagent used during the cleavage is an acid mixture containing 81.5% of TFA and phenol scavengers (5%), thioanisol (5%), water (5%), ethanediol (2.5%)  
10 and triisopropylsilane (1%). The resin was treated with this mixture for three hours with stirring and at ambient temperature, in a proportion of 100 ml of solution per gram of resin. The free peptide in solution was recovered by filtration. The peptide was  
15 then precipitated and washed under cold conditions in diisopropyl ether and then dissolved in 20% acetic acid and lyophilized.

The peptide recovered after lyophilization, the  
20 synthesis crude, is in reduced form, i.e. the intrachain disulphide bridges are not formed. The formation of these covalent bonds was performed using the cystamine/cysteamine redox couple. The synthesis crude was taken up in water to which 0.1% (v/v) of TFA  
25 and 6 M guanidinium chloride had been added in order to facilitate the dissolving thereof, in a proportion of 2.0 mg.ml<sup>-1</sup>. This solution was then added, dropwise, diluted to 0.2 mg/ml<sup>-1</sup>, to the reducing buffer, made up of 100 mM Tris/HCl, pH 7.8, and 5 mM cysteamine.  
30 Cystamine (oxidizing agent), at a final concentration of 0.5 mM, was added after 45 minutes of reaction at ambient temperature. The medium was brought to pH 3.0 after 30 minutes.

35 The cysteamine makes it possible to reduce the thiol groups present on the peptide. In the open air, it oxidizes and allows the oxidation of cysteines and



therefore the folding of the peptide by formation of intrachain disulphide bridges. The cystamine added at the end of the manipulation makes it possible to complete the folding. The correct progress of the  
5 oxidation is verified by analytical chromatography, comparing the retention times of the crude and oxidized products, which are greater for the former.

The peptides were purified by reverse-phase high  
10 performance liquid chromatography on a Vydac C18 (1.0 x 25.0 cm) preparative column. A linear gradient of 0-60% acetonitrile in a 0.1% aqueous trifluoroacetic acid solution, over 90 minutes, was used. The fractions of the major peak were analysed by analytical HPLC; the  
15 fractions exhibiting just one peak were combined and lyophilized.

The products thus obtained were analysed by mass spectrometry. They are the peptides of the sequence  
20 listing attached in the appendix.

EXAMPLE 2: SYNTHESIS OF POLYANION OF THE HEPARIN OR HEPARAN SULPHATE TYPE, WHICH CAN BE USED FOR THE COMPOSITION OF THE PRESENT INVENTION

25 A) Enzymatic synthesis

A molecule of heparin or of heparan sulphate having a defined degree of polymerization dp is synthesized.

30 6 g of heparin are solubilized in a buffer containing 5 mM of Tris, 2 mM of  $\text{CaCl}_2$ , 50 mM of NaCl and 0.1 mg/ml of albumin. The pH is adjusted to 7.5 with acetic acid. This solution is incubated at 25°C with heparinase I (8 mU/ml) for approximately 50 h (the  
35 enzymatic reaction is monitored by means of the increase in optical density, measured at 232 nm).

The mixture is then purified by gel filtration chromatography. The solid phase is Biogel P10, contained in a 1.50 m column 4.4 cm in diameter, eluted at 1 ml/min with 0.25 M NaCl.

5

Figure 11 represents the absorbance at 230 nm of the various fractions of 15 ml obtained for degrees of polymerization dp ranging from 2 to 10.

10 The various oligosaccharides (dp2, dp4, etc.) are dialysed against water and then lyophilized.

B) Synthesis by chemical depolymerization from the natural product

15

When the starting material is heparin, the following procedures are carried out: 1 g of heparin is solubilized in 20 ml of sodium nitrite ( $\text{NaNO}_2$ ) at 2.1 mg/ml. The solution is adjusted to pH 1.5 with sulphuric acid, and is then incubated at 4°C for 3 h. The reaction is stopped and the oligosaccharides are purified as above in paragraph A).

When the starting material is heparan sulphate, the following procedure is carried out: 8 g of heparan sulphate are solubilized in 40 ml containing 5 mM of Tris, 2 mM of  $\text{CaCl}_2$ , 50 mM of NaCl and 0.1 mg/ml of albumin. The pH is adjusted to 7.5 with acetic acid. This solution is incubated at 30°C with heparinase III (25 mU/ml) for approximately 72 h. Heparinase III is again added, for a period of 48 h, and the products are then purified as described above in paragraph A).

EXAMPLE 3: SYNTHESIS OF A COMPOSITION OF THE PRESENT INVENTION: MIXTURE OF A CD4 PEPTIDE WITH A POLYANION

35

In this example, a CD4 peptide of Example 1 is mixed

with a heparan sulphate prepared in Example 2.

These two molecules are dissolved at a concentration that is two times the desired final concentration.

5 These solubilizations are carried out in a physiological buffer; for example, PBS, TBS (50 mM Tris, 0.15 M NaCl, pH 7.5) or HBS (20 mM Hepes, 0.15 M NaCl, pH 7.5).

10 The two preparations are then mixed volume for volume (1/1).

EXAMPLE 4: SYNTHESIS OF A COMPOSITION OF THE PRESENT INVENTION: COUPLING OF A CD4 PEPTIDE WITH A POLYANION

15

In this example, a CD4 peptide of Example 1 is coupled with the heparan sulphate prepared in Example 2.

20 The heparan sulphate is incubated with a molar excess of hydrazine or of carbodihydrazide. The function of this step is to place a hydrazine group on the reductive end of the oligosaccharide, when it is prepared by enzymatic depolymerization, or on the aldehyde of the oligosaccharide, when it is prepared by  
25 chemical depolymerization with nitrous acid.

The carbohydrates of the soluble CD4 peptide are oxidized by treatment with sodium periodate, and the aldehyde function thus created is used for the coupling  
30 of the hydrazine-containing oligosaccharide.

The oligosaccharide, generally in solution at 1 mM in PBS buffer (sodium phosphate saline), is coincubated with a molar excess (for example up to 100 times) of  
35 hydrazine or of carbodihydrazide, also in solution in PBS. The reaction mixture is incubated at ambient temperature, then purified by desalification or

dialysis against distilled water and, finally, dried by evaporation under vacuum or lyophilized.

5 The glycosylated (produced in mammalian cells or insect cells) soluble CD4 molecule (sCD4) is taken up in a 20 mM phosphate buffer, pH 6.2, and then treated with sodium periodate (10 mM) for 20 minutes at 4°C and in the dark. To remove the sodium periodate, the reaction mixture is desalified by gel filtration or by dialysis  
10 against the phosphate buffer.

The sCD4, the glycans of which are thus oxidized, is coincubated with a molar excess of hydrazine-containing oligosaccharide at 4°C, so as to form a complex between  
15 the two molecules.

When the CD4 is not glycosylated, the procedure is carried out in the manner described by Najjam et al., in document [17].  
20

It is also possible to use any type of bridging agent which binds, firstly, to one end of the oligosaccharide and, secondly, to a part of the CD4 peptide that is not essential to its function. Those skilled in the art  
25 will have no difficulty in implementing this process or an equivalent process.

EXAMPLE 5: DEMONSTRATION OF THE INCREASE IN THE AFFINITY OF gp120 FOR HEPARIN BY MEANS OF CD4

30 30 resonance units (RU) of biotinylated heparin are immobilized at the surface of a biochip ("sensorchip" B1 produced by the company Biacore).

35 gp120 (hxbc2) at 50 nM is incubated for 1 hour 30 min with increasing concentrations of soluble CD4 at 0, 50, 100, 250 or 500 nM, and then injected onto the heparin

surface at 10  $\mu$ l/min.

The analyses of gp120-heparin interaction by surface plasmon resonance were carried out as a function of  
5 time.

The curves in Figure 1 correspond to the injection of gp120 at 50 nM and of CD4 respectively at 0, 50, 100, 250 or 500 nM (respectively for the curves from bottom  
10 to top in this figure).

Figure 2 shows the amount of gp120/CD4 bound to the heparin as a function of the concentration of CD4.

15 It appears that a CD4:gp120 molar ratio of approximately 5:1 produces the maximum response.

These results show that exposure of the CD4i domain of gp120 greatly increases the interaction of gp120 with  
20 heparin. CD4i therefore represents a new site of interaction with heparin.

#### EXAMPLE 6: CD4-DEPENDENT gp120/48D INTERACTION

25 1250 RU of antibody 48d specific for the CD4i epitope are immobilized at the surface of a biochip ("sensorchip" B1) as in Example 1 above.

The gp120 viral protein (hxbc2) at 50 nM is incubated  
30 for 1 h 20 min with increasing concentrations of soluble CD4 at 0, 50, 100, 240 or 500 nM, and then injected onto the 48d surface at 10  $\mu$ l/min.

The analyses of gp120-heparin interaction by surface  
35 plasmon resonance were carried out as a function of time.

The curves in Figure 3 correspond to the injection of gp120 at 50 nM and of CD4 respectively at 0, 50, 100, 250 or 500 nM (respectively for the curves from bottom to top in this figure).

5

This example shows that the gp120/48d interaction is CD4-dependent, and that 48d interacts with CD4i, the coreceptor recognition domain. This antibody can therefore be used as a model for the interaction of gp120 with a coreceptor.

10

EXAMPLE 7: INHIBITION OF THE gp120 PROTEIN-48d INTERACTION BY HEPARIN

15 The gp120 protein is coincubated for 40 minutes with CD4. The mixture is then divided up into 5 aliquots, to which the heparin (15 kDa) is added at various concentrations.

20 The final concentrations in the aliquots are: gp120 : 50 nM; CD4 : 250 nM and heparin : 0, 3, 6, 12 or 30 µg/ml, respectively, from top to bottom in Figure 4. On the top curve, where there is no heparin, the gp120/CD4 interaction is visualized; all the other curves are  
25 in the presence of heparin (from 3 to 30 µg/ml, respectively, from top to bottom, Figure 4).

After incubation for 40 minutes, the various mixtures are injected onto the 48d surface.

30

The analyses of gp120-heparin interaction by surface plasmon resonance were carried out as a function of time.

35 The results obtained are represented in Figure 4. They show that heparin inhibits the adsorption of the gp120/CD4 complex onto the 48d-antibody surface. The

heparin is, moreover, found to be an effective inhibitor since the inhibition is virtually complete from the lowest of the concentrations tested (3  $\mu$ g/ml).

5 This shows that the heparin competes with 48d and therefore binds to CD4i.

This example indicates that the inhibitory activity of the oligosaccharides (heparin), as defined above, is  
10 obtained in the presence of CD4.

This result makes it possible to propose the use of a hybrid molecule made up of CD4 and of oligosaccharides of the heparin type, linked covalently, or of a mixture  
15 of these two molecules.

The direct interaction of the CD4i domain with a polyanion has never been described in the prior art, neither has inhibition of the gp120-antibody 48d  
20 interaction by a polyanion. No studies existed showing the possible inhibition of gp120 with the coreceptors by means of a molecule of the heparin type.

EXAMPLE 8: INHIBITION OF THE gp120-48d INTERACTION WITH  
25 OLIGOSACCHARIDE FRAGMENTS OF DEFINED SIZES

The oligosaccharide fragments of defined sizes are obtained by enzymatic depolymerization.

30 The gp120 viral protein is coincubated for 60 minutes with CD4 so as to expose the CD4i domain. The mixture is divided up into 8 aliquots and heparin fragments of increasing size, comprising from 1 to 8 basic disaccharide units, i.e. a degree of polymerization  
35 (dp) of 2 to 16, are added so as to give final concentrations of 50 nM for gp120, 250 nM for CD4 and 125 nM for the heparin fragments (the molecular mass of

never been described elsewhere and constitutes the first proof of a possible interaction between gp120 and heparin or heparan sulphate via the CD4i site.

5 A molecular modelling study showed that the CD4i site of gp120 consists of basic amino acids. These basic amino acids are aligned on the surface of the protein, and effectively constitute a site for interaction with heparin, or oligosaccharides derived from heparin or  
10 from heparan sulphate.

The inventors therefore hereby propose a therapeutic use of the polyanionic compounds targeting this novel site of interaction. The approach consists of the  
15 conjugated use of polyanions and of molecules capable of exposing the CD4i epitope, by coadministration or in the form of a hybrid molecule. This type of molecule simultaneously blocks all the domains of interaction of gp120 with the host cells.

20



Chemokine receptors-future therapeutic targets for HIV?  
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25

**CLAIMS**

1. Composition characterized in that it comprises a polyanion and a molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein.  
5
2. Composition according to Claim 1, in which the polyanion is chosen from the group consisting of heparin, heparan sulphate, and a polyanion equivalent to heparin or to heparan sulphate.  
10
3. Composition according to Claim 2, in which the heparin, the heparan sulphate or the polyanion equivalent to heparin or to heparan sulphate has a degree of polymerization dp of 10 to 24.  
15
4. Composition according to Claim 2, in which the heparin, the heparan sulphate or the polyanion equivalent to heparin or to heparan sulphate has a degree of polymerization dp of 12 to 20.  
20
5. Composition according to Claim 2, in which the heparin, the heparan sulphate or the polyanion equivalent to heparin or to heparan sulphate has a degree of polymerization dp of 15 to 17.  
25
6. Composition according to Claim 1, in which the molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein is a CD4 peptide or a derivative of this peptide.  
30

## CLAIMS

1. Composition characterized in that it comprises a polyanion and a molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein chosen from a CD4 peptide or a derivative of this peptide, or else a monoclonal antibody which binds to the gp120 viral protein and which is capable of activating said gp120 protein in a manner equivalent to the CD4 peptide.
2. Composition according to Claim 1, in which the polyanion is chosen from the group consisting of heparin, heparan sulphate, and a polyanion equivalent to heparin or to heparan sulphate.
3. Composition according to Claim 2, in which the heparin, the heparan sulphate or the polyanion equivalent to heparin or to heparan sulphate has a degree of polymerization dp of 10 to 24.
4. Composition according to Claim 2, in which the heparin, the heparan sulphate or the polyanion equivalent to heparin or to heparan sulphate has a degree of polymerization dp of 12 to 20.
5. Composition according to Claim 2, in which the heparin, the heparan sulphate or the polyanion equivalent to heparin or to heparan sulphate has a degree of polymerization dp of 15 to 17.

7. Composition according to Claim 6, in which the CD4 peptide has the sequence (I) below:

5 Cys or TPA - P<sup>1</sup> - Cys - P<sup>2</sup> - Cys - P<sup>3</sup> - Cys - Ala or Gln  
- Gly or (D)Asp or Ser - Ser or His or Asn - Xaa<sup>J</sup> - Cys  
- Thr or Ala - Cys - Xaa<sup>K</sup> - NH<sub>2</sub>

10 in which TPA represents thiopropionic acid, Xaa<sup>J</sup>  
represents  $\beta$ -naphthylalanine, phenylalanine or  
biphenylalanine, Xaa<sup>K</sup> represents Gly, Val or Ileu, P<sup>1</sup>  
represents 3 to 6 amino acids, P<sup>2</sup> represents 2 to 4  
amino acids and P<sup>3</sup> represents 6 to 10 amino acids, the  
15 amino acids in P<sup>1</sup>, P<sup>2</sup> and P<sup>3</sup> being natural or unnatural,  
identical or different, and P<sup>1</sup>, P<sup>2</sup> and P<sup>3</sup> possibly  
having a common sequence, said peptide having a  
 $\beta$ -hairpin conformation in which the  $\beta$ -turn is made up  
of the amino acid residues Ala or Gln - Gly or DAsp or  
Ser-Ser or His or Asn- Xaa<sup>J</sup> of its sequence (A).

20

8. Composition according to Claim 6, in which the CD4 peptide is chosen from the sequences ID No. 1 to ID No. 18 of the sequence listing attached in the appendix.

25 9. Composition according to any one of Claims 1 to 8,  
in which the polyanion and the molecule capable of  
inducing the exposure of the CD4i epitope of the gp120  
viral protein are mixed in said composition.

6. Composition according to Claim 1, in which the CD4 peptide has the sequence (I) below:

5

Cys or TPA - P<sup>1</sup> - Cys - P<sup>2</sup> - Cys - P<sup>3</sup> - Cys - Ala or Gln  
- Gly or (D)Asp or Ser - Ser or His or Asn - Xaa<sup>J</sup> - Cys  
- Thr or Ala - Cys - Xaa<sup>K</sup> - NH<sub>2</sub>

10 in which TPA represents thiopropionic acid, Xaa<sup>J</sup>  
represents  $\beta$ -naphthylalanine, phenylalanine or  
biphenylalanine, Xaa<sup>K</sup> represents Gly, Val or Ileu, P<sup>1</sup>  
represents 3 to 6 amino acids, P<sup>2</sup> represents 2 to 4  
15 amino acids and P<sup>3</sup> represents 6 to 10 amino acids, the  
amino acids in P<sup>1</sup>, P<sup>2</sup> and P<sup>3</sup> being natural or unnatural,  
identical or different, and P<sup>1</sup>, P<sup>2</sup> and P<sup>3</sup> possibly  
having a common sequence, said peptide having a  
 $\beta$ -hairpin conformation in which the  $\beta$ -turn is made up  
20 of the amino acid residues Ala or Gln - Gly or DAsp or  
Ser-Ser or His or Asn- Xaa<sup>J</sup> of its sequence (A).

7. Composition according to Claim 1, in which the CD4 peptide is chosen from the sequences ID No. 1 to ID No. 18 of the sequence listing attached in the appendix.

25

8. Composition according to any one of Claims 1 to 7, in which the polyanion and the molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein are mixed in said composition.

30

10. Composition according to Claim 9, in which the polyanion and the molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein are mixed in said composition in proportions of 1 to 10 mol of polyanion per 0.5 to 1.5 mol of molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein.

11. Composition according to Claim 8, in which the polyanion and the molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein are mixed in said composition in proportions of 5 mol of polyanion per mole of molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein.

12. Composition according to any one of Claims 1 to 8, in which the polyanion and the molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein are linked to one another in said composition.

13. Composition according to Claim 12, in which the polyanion and the molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein are linked to one another at one of the ends of the polyanion.

14. Composition according to Claim 12, in which the polyanion and the molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein

9. Composition according to Claim 8, in which the polyanion and the molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein are mixed in said composition in proportions of 1 to 5 10 mol of polyanion per 0.5 to 1.5 mol of molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein.

10. Composition according to Claim 7, in which the 10 polyanion and the molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein are mixed in said composition in proportions of 5 mol of polyanion per mole of molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral 15 protein.

11. Composition according to any one of Claims 1 to 7, in which the polyanion and the molecule capable of inducing the exposure of the CD4i epitope of the gp120 20 viral protein are linked to one another in said composition.

12. Composition according to Claim 11, in which the polyanion and the molecule capable of inducing the 25 exposure of the CD4i epitope of the gp120 viral protein are linked to one another at one of the ends of the polyanion.

13. Composition according to Claim 11, in which the 30 polyanion and the molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein



- 34 -

are linked to one another by means of a spacer arm of the polyethylene glycol type.

14. Method for producing a composition according to  
5 Claim 9, comprising the following steps:

- preparing the polyanion,
- preparing the molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein,
- 10 - mixing the polyanion and the molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein prepared so as to obtain said composition.

15 15. Method for producing a composition according to Claim 12, comprising the following steps:

- preparing the polyanion,
- preparing the molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein,
- 20 - linking the polyanion and the molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein prepared so as to obtain said composition.

25 16. Method of production according to Claim 14 or 15, in which the polyanion is prepared by partial depolymerization of heparin or of heparan sulphate by means of an enzymatic or chemical method.

30

35

are linked to one another by means of a spacer arm of the polyethylene glycol type.

5 14. Method for producing a composition according to Claim 8, comprising the following steps:

- preparing the polyanion,
- preparing the molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein,
- 10 - mixing the polyanion and the molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein prepared so as to obtain said composition.

15

15. Method for producing a composition according to Claim 11, comprising the following steps:

- preparing the polyanion,
- preparing the molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein,
- 20 - linking the polyanion and the molecule capable of inducing the exposure of the CD4i epitope of the gp120 viral protein prepared so as to obtain said composition.

25

16. Method of production according to Claim 14 or 15, in which the polyanion is prepared by partial depolymerization of heparin or of heparan sulphate by  
30 means of an enzymatic or chemical method.

17. Method of production according to Claim 14 or 15,  
in which, since the molecule capable of inducing the  
exposure of the CD4i epitope of the gp120 viral protein  
5 is a peptide, it is prepared by solid-phase chemical  
synthesis or by genetic recombination.

18. Use of a composition according to any one of  
Claims 1 to 8, for preparing a medicinal product.

10

19. Use of a composition according to any one of  
Claims 1 to 8, for preparing a medicinal product  
intended for the treatment of AIDS.

17. Method of production according to Claim 14 or 15,  
in which, since the molecule capable of inducing the  
exposure of the CD4i epitope of the gp120 viral protein  
is a peptide, it is prepared by solid-phase chemical  
5 synthesis or by genetic recombination.

18. Use of a composition according to any one of  
Claims 1 to 7, for preparing a medicinal product.

10 19. Use of a composition according to any one of  
Claims 1 to 7, for preparing a medicinal product  
intended for the treatment of AIDS.

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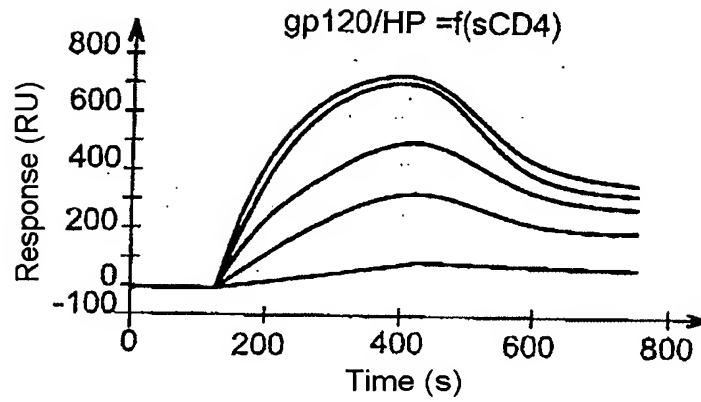


FIG. 1

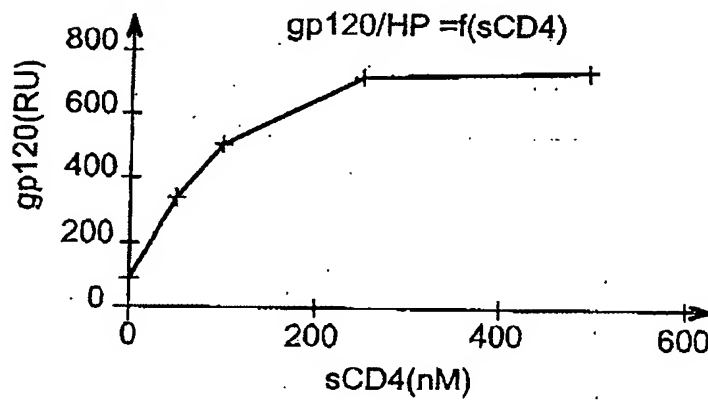


FIG. 2

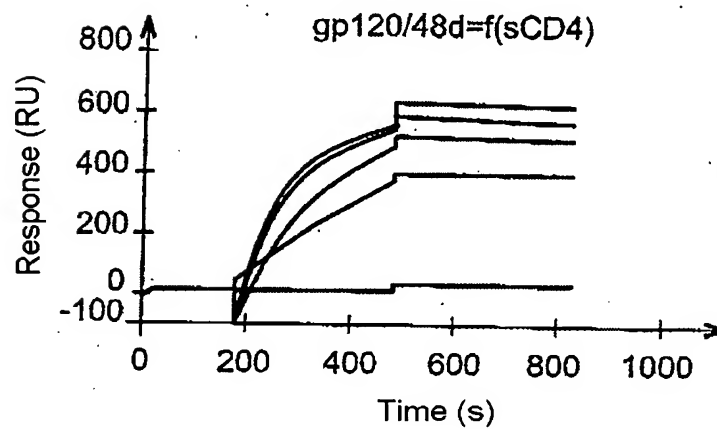


FIG. 3

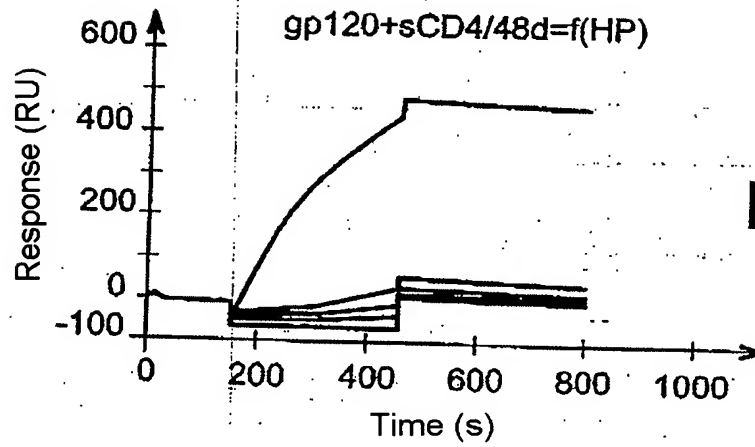


FIG. 4

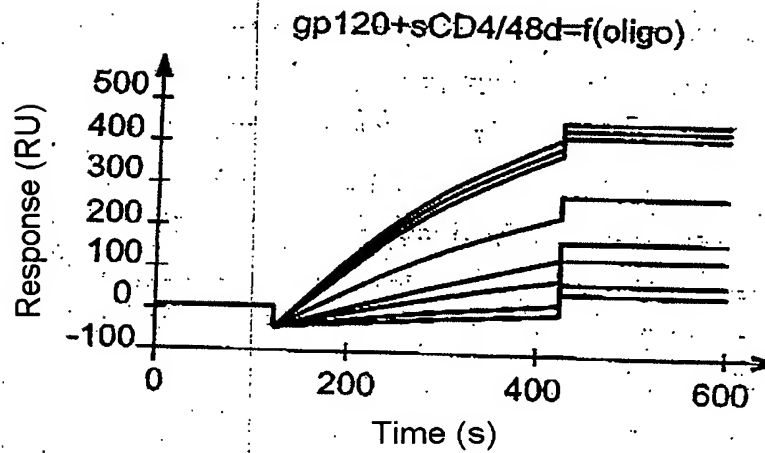


FIG. 5

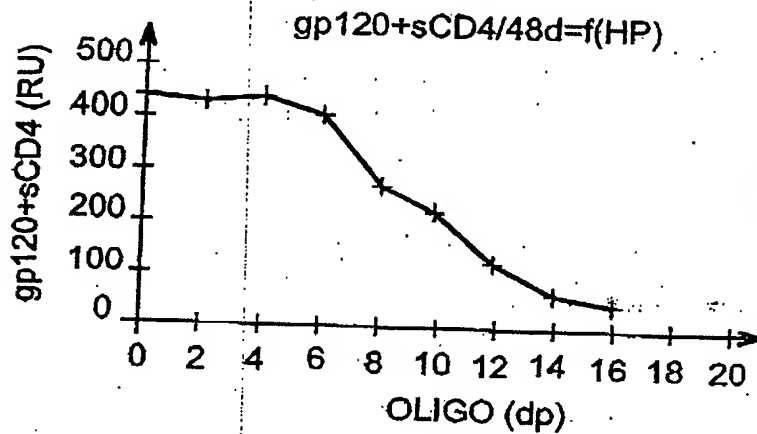


FIG. 6

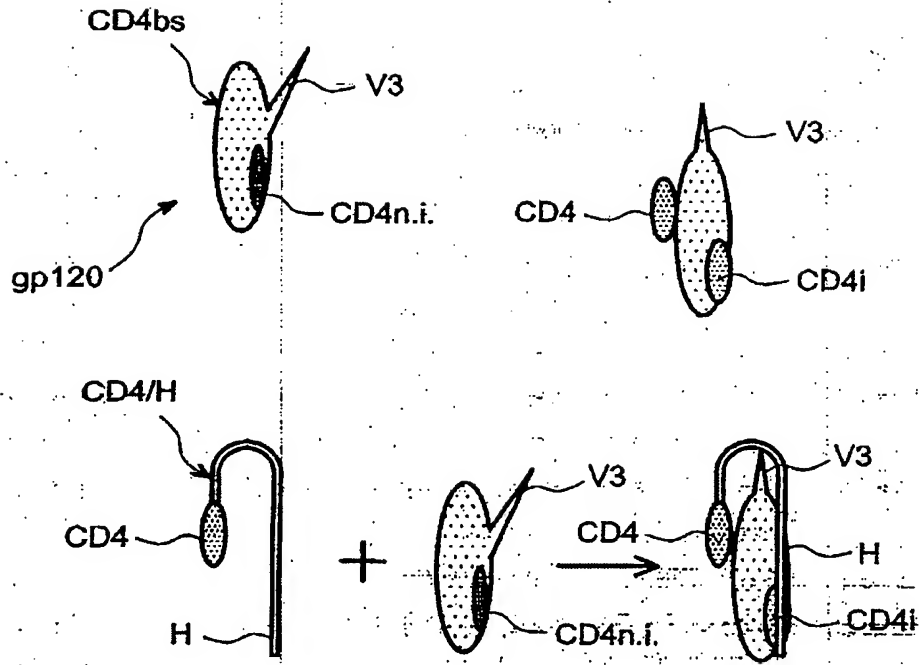


FIG. 7

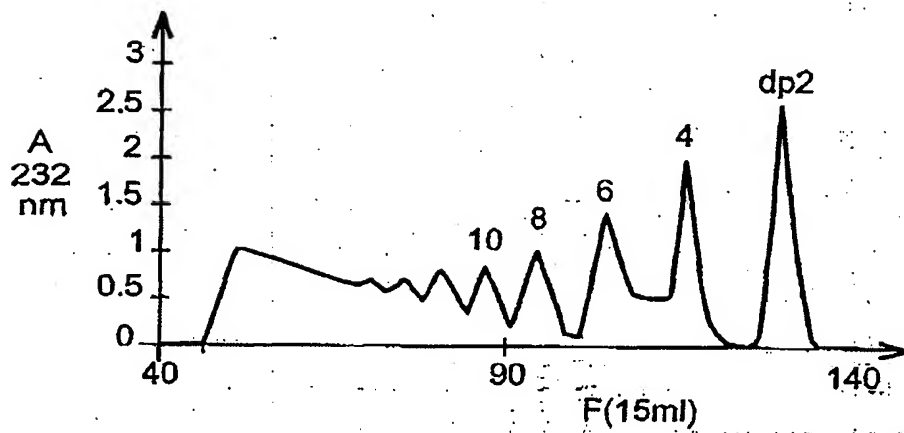


FIG. 11

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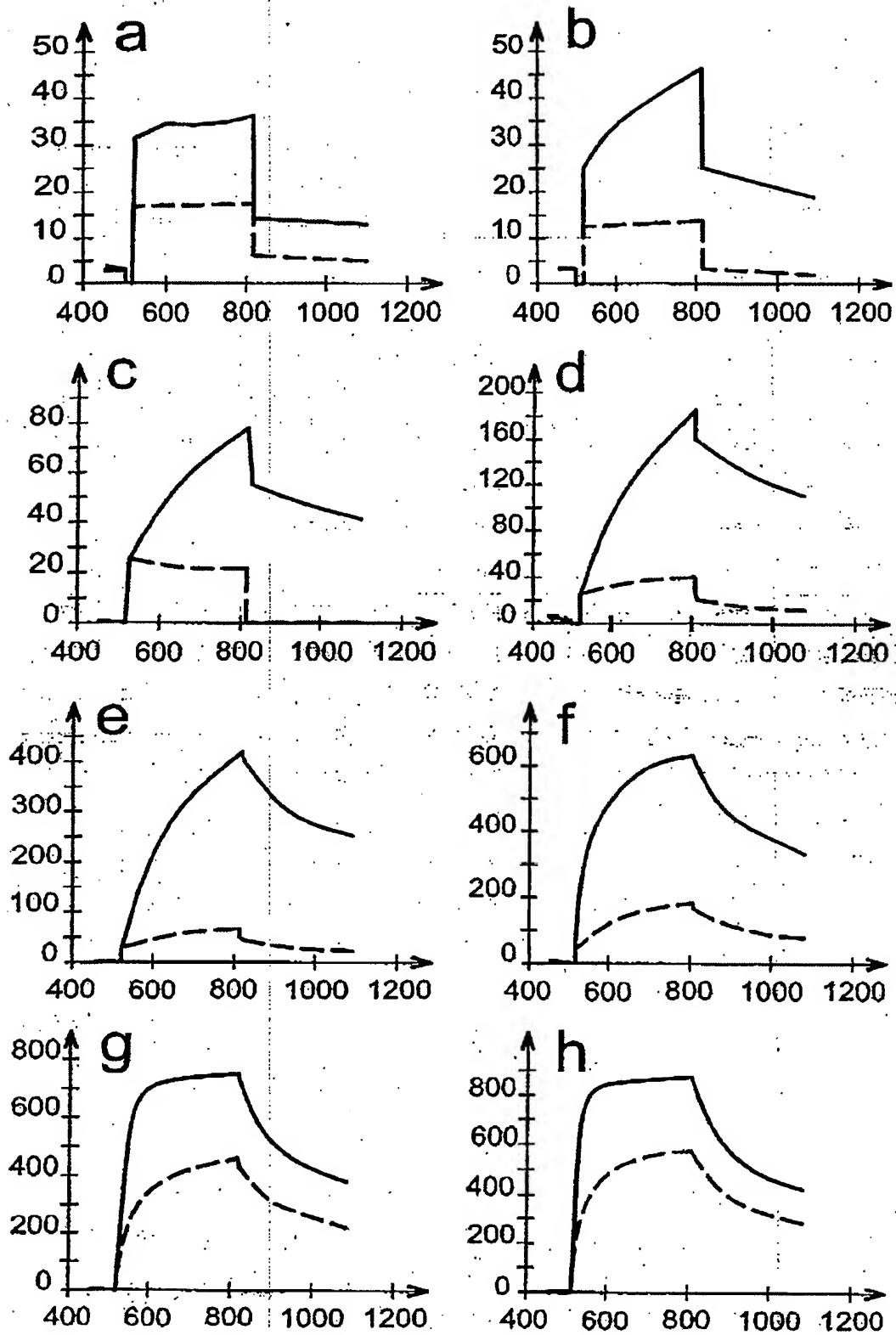


FIG. 8



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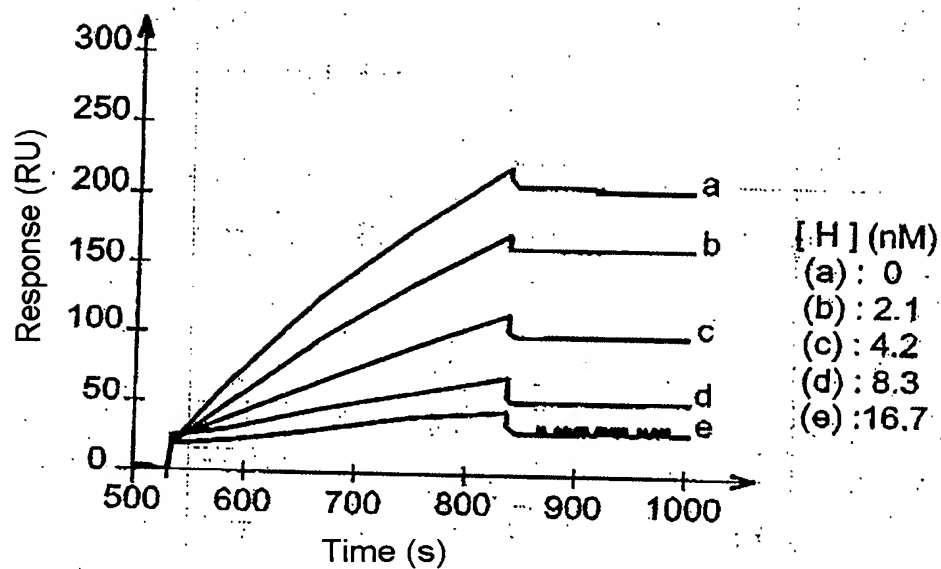


FIG. 9

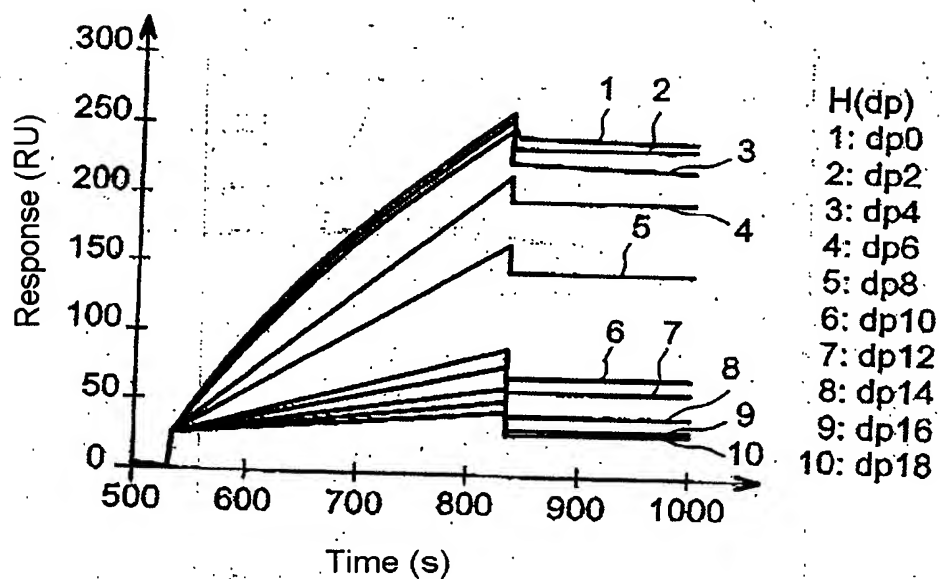


FIG. 10

SEQUENCE LISTING

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<120> ANTI-HIV COMPOSITION, METHOD OF PRODUCTION AND MEDICINAL PRODUCT

<130> B13987.3 EE

<140>

<141>

<160> 18

<170> PatentIn Ver. 2.1

<210> 1

<211> 16

<212> PRT

<213> Homo sapiens

<220>

<223> Sequence Gln33 to Pro48 of human CD4

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<211> 31

<212> PRT

<213> scorpion

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sequence derived from scyllatoxin

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Gly Lys Cys Ala Gly Ser Phe Cys Ala Cys Gly Pro  
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Gly Lys Cys Ala Gly Asn Phe Cys Ala Cys Val  
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1				5				10						15	

Gly	Lys	Cys	Ala	Xaa	Ser	Xaa	Cys	Ala	Cys	Ile
			20				25			

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Xaa	Asn	Leu	His	Phe	Cys	Val	Gln	Arg	Cys	His	Ser	Leu	Gly	Lys	Leu
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Gly	Lys	Cys	Ala	Gly	Ser	Phe	Cys	Ala	Cys	Val
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sequence derived from scyllatoxin

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sequence derived from scyllatoxin

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20 25 30

Pro